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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

92201252.1

PRIORITY DOCUMENT

R. C. SMITH

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

Den Haag, den
The Hague, 01/03/93
La Haye, le

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Date of filing: 05/05/92
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Anmelder:
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NETHERLANDS

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Title of the invention:
Titre de l'invention: Peptides of human papilloma virus for use in human T cell response inducing compositions

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Peptides of Human Papilloma Virus for use in human T cell response inducing compositions

Field of the invention

The invention is concerned with novel peptides derived from Human Papilloma Virus proteins and their use in pharmaceutical compositions for a prophylactic or therapeutic treatment of human individuals against Human Papilloma Virus-related diseases such as cervical cancer.

Background of the invention

Human Papilloma Viruses (HPVs) are implicated in the etiology of cervical cancer, the fifth most common cancer worldwide and the second cause of cancer-related death in women. If other HPV-related cancers are counted also, up to 10% of the worldwide mortality due to cancer is linked to HPVs. HPVs are double stranded circular DNA viruses of about 8 kilobases. Until now more than 60 genotypes have been described of which several are associated with cancer.

HPV-DNA can be found in cervical dysplastic lesions and in cervical carcinomas in which the percentage of HPV positivity increases up to 99% when the lesions progress towards malignancy. The most important HPV types associated with cervical carcinoma are HPV16 and 18 of which HPV16 alone accounts for more than 50% of the HPV positive cervical carcinomas.

The DNAs of several HPVs have been sequenced. The DNA open reading frames can be divided into early regions (E) and late regions (L). The E regions are coding for proteins needed for virus replication and transformation. The L regions encode viral capsid proteins. The E6 and E7 proteins are involved in the pathogenesis of HPV-induced abnormal cell proliferation and these

genes are expressed in tissue or tumor cells obtained from cervical cancers associated with HPV infection.

In addition, the HPV16 E6 and E7 genes are capable of inducing epithelial cell transformation in the cell culture without the presence of other HPV genes indicating that at least part of the stimulation of cell proliferation caused by HPV infection is due to the E6 and E7 viral proteins.

Cytotoxic T lymphocytes (CTL) are of crucial importance in the resistance against virus infections and the immune surveillance against virus-induced tumors (reviewed in 1). CTL specific for viruses or virus-induced tumors recognize short viral protein-derived peptides, of about 9 amino acids in length, that are bound to the antigen presenting groove of major histocompatibility complex (MHC) class I molecules (reviewed in 1). Recently in several virus systems vaccination with peptides recognized by antigen-specific CTL was shown to prevent lethal virus infections and to delay tumor growth in mice (reviewed in 1 and 2).

We have succeeded in the identification of viral peptides that bind to the groove of MHC class I molecules by using the antigen processing defective cell line 174.CEM T2 generated and provided by P. Cresswell (3). This cell line expresses the human MHC class I HLA-A2.1 and HLA-B5 alleles of which only the HLA-A2.1 molecules are expressed as partly empty and unstable molecules that can be stabilized on the cell surface with exogenously added peptides. If incubation with peptide results in an increase in the cell surface expression of this MHC molecule, this implies that the peptide binds to the groove of the HLA-A2.1 molecule and is therefore a possible candidate to be recognized by CTL. The HLA-A2.1 molecule is the most frequent HLA molecule present in the Western European Caucasoid population. About 50% of this population expresses this allele.

Using the amino acid sequence of the E6 and E7 proteins of HPV16 (4) we generated all possible nonapeptides (9 amino acid long peptides) overspanning the entire E6 and E7 region. Every amino acid was used as a start amino acid for these 9-mer peptides. Every peptide was individually tested with respect to

its capacity to bind to the HLA-A2.1 molecule. In total 10 peptides in the HPV16 E6 region and 6 in the HPV16 E7 region were identified to bind to the HLA-A2.1 molecule. This implies that all important candidate peptides of HPV16 for use as a vaccine in HLA-A2.1 positive humans have been identified.

The purpose of the present invention is therefore to provide specific synthetic peptides for prevention, prophylaxis, therapy and treatment of cervical carcinoma and other HPV16-related diseases utilizing these synthetic peptides and pharmaceutical compositions containing the synthetic peptides.

Summary of the invention

The present invention provides specific peptides derived from the amino acid sequence of the HPV16 E6 and E7 region which, because of their capability to bind to the HLA-2.1 protein, are candidate peptides to be included in human vaccines that can induce protective or therapeutic T cell responses against HPV16.

The novel peptides of the present invention are useful in pharmaceutical compositions, as screening tools and in the prevention, prophylaxis, therapy and treatment of HPV16-induced diseases or other conditions which would benefit from inhibition of HPV16 infection.

This invention provides a peptide comprising an amino acid sequence derived from a protein of human papilloma virus (HPV), wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex (MHC) Class I molecule.

In a preferred embodiment of the invention, said amino acid sequence is derived from protein E6 or E7 of HPV16.

Preferably, said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1.

More specifically, this invention provides a peptide comprising an amino acid sequence derived from protein E6 or E7 of HPV16, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1 and is selected from the group consisting of:

1. KLPQLCTEL (residues 18-26 of HPV16 protein E6)
2. QLCTELQTT (residues 21-29 of HPV16 protein E6)
3. LCTELQTTI (residues 22-30 of HPV16 protein E6)
4. ELQTTIHDI (residues 25-33 of HPV16 protein E6)
5. LQTTIHDII (residues 26-34 of HPV16 protein E6)
6. TIHDIILEC (residues 29-37 of HPV16 protein E6)
7. IHDIILECV (residues 30-38 of HPV16 protein E6)
8. CVYCKQQLL (residues 37-45 of HPV16 protein E6)
9. KISEYRHYC (residues 79-87 of HPV16 protein E6)
10. PLCDLLIRC (residues 102-110 of HPV16 protein E6)
11. TLHEYMLDL (residues 7-15 of HPV16 protein E7)
12. YMLDLQPET (residues 11-19 of HPV16 protein E7)
13. MLDLQPETT (residues 12-20 of HPV16 protein E7)
14. TLEDLLMGT (residues 78-86 of HPV16 protein E7)
15. GTLGIVCPI (residues 85-93 of HPV16 protein E7)
16. TLGIVCPIC (residues 86-94 of HPV16 protein E7)
17. a fragment, homolog, isoform, derivative, genetic variant or conservative variant of any one of the amino acid sequences Nos. 1-16 which has the ability to bind to human MHC Class I allele HLA-A2.1.

This invention further provides a pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to the invention, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. Preferably, said pharmaceutical composition contains a peptide according to the invention which is able to induce a T cell response effective against HPV.

In addition, this invention provides a method of prophylactic or therapeutic treatment of cervical carcinoma and other HPV-related diseases with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a peptide according to the invention, more specifically an immunogenic form of a peptide according to the invention which is able to induce a T cell response effective against HPV.

Detailed description of the invention

The invention is directed to peptides comprising an amino acid sequence derived from a protein of HPV, wherein said amino acid sequence has the ability to bind to a human MHC Class I molecule. A most preferred embodiment of the invention concerns peptides comprising an amino acid sequence derived from protein E6 or E7 of HPV16, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1. Specifically, such peptides comprise the following amino acid sequences derived from the noted regions of HPV16 (see Table I; the amino acids are identified by the one-letter code of amino acids).

TABLE I

Peptide	Amino acid sequence	HPV16 region
1	KLPQLCTEL	E6 (residues 18 - 26)
2	QLCTELQTT	E6 (residues 21 - 29)
3	LCTELQTTI	E6 (residues 22 - 30)
4	ELQTTIHDI	E6 (residues 25 - 33)
5	LQTTIHDII	E6 (residues 26 - 34)
6	TIHDIILEC	E6 (residues 29 - 37)
7	IHDIILECV	E6 (residues 30 - 38)
8	CVYCKQQLL	E6 (residues 37 - 45)
9	KISEYRHYC	E6 (residues 79 - 87)
10	PLCDLLIRC	E6 (residues 102-110)
11	TLHEYMLDL	E7 (residues 7 - 15)
12	YMLDLQPET	E7 (residues 11 - 19)
13	MLDLQPETT	E7 (residues 12 - 20)
14	TLEDLLMGT	E7 (residues 78 - 86)
15	GTLGIVCPI	E7 (residues 85 - 93)
16	TLGIVCPIC	E7 (residues 86 - 94)

The data suggest that peptides 1-16 are single polypeptides of identified sequences. However, homologs, isoforms or genetic variants of these peptides 1-16 may exist within or outside the cellular environment. This invention encompasses all such homologs, isoforms or genetic variants of peptides 1-16 provided that they bind to the HLA-A2.1 molecule. Polypeptides that are homologs of peptides 1-16 specifically include those having amino acid sequences which are at least about 40% conserved in relation to the amino acid sequence set forth in Table I, preferentially at least about 60% conserved, and more preferentially at least about 75% conserved.

It will be understood by one of ordinary skill in the art that other variants of peptides 1-16 are included within the scope of the present invention. This particularly includes any variants that differ from the synthesized peptides 1-16 only by conservative amino acid substitution. In particular, replacements of C (cysteine) by A (alanine), S (serine), α-aminobutyric acid and others are included as it is known that cysteine-containing peptides are susceptible to (air) oxidation during synthesis and handling. Many such conservative amino acid substitutions are set forth as sets in (5).

Herein, peptides 1-16 or fragments thereof include any variation in the amino acid sequence, whether by conservative amino acid substitution, deletion, or other processes, provided that the polypeptides bind to the HLA-A2.1 molecule. The fragments of peptides 1-16 may be small peptides with sequences of as little as 5 or more amino acids, said sequences being those disclosed in Table I when said polypeptides bind to the HLA-A2.1 molecule.

Polypeptides larger than peptides 1-16 are especially included within the scope of the present invention when said polypeptides induce a HPV16-specific CTL response in HLA-A2.1 positive individuals and include a (partial) amino acid sequence as set forth in Table I, or conservative substitutions thereof. Such polypeptides may have a length up to about 30 amino acids, preferably up to about 27 amino acids.

This invention includes the use of polypeptides 1-16 generated by every means, whether genetic engineering, peptide synthesis with solid phase techniques or others. The foregoing peptides 1-16 may have various chemical modifications made at the terminal ends and still be within the scope the present invention. Also other chemical modifications are possible, particularly cyclic and dimeric configurations. The term "derivatives" intends to cover all such modified peptides.

The polypeptides of the present invention find utility for the treatment or prevention of diseases involving HPV16 such as genital warts, cervical cancer or others that are linked to HPV16.

For all applications the peptides are administered in an immunogenic form. Since the peptides are relatively short, this may necessitate conjugation with an immunogenicity conferring carrier material such as lipids or others or the use of adjuvants.

The magnitude of a prophylactic or a therapeutic dose of polypeptides 1-16 of this invention will, of course, vary with the group of patients (age, sex, weight, etcetera), the nature of the severity of the condition to be treated, the particular polypeptide of this invention and its route of administration. Any suitable route of administration may be employed to achieve an effective dosage of a polypeptide identified by this invention, as well as any dosage form well known in the art of pharmacy. In addition the polypeptides 1-16 may also be administered by controlled release means and/or delivery devices. They may also be administered in combination with other active substances, such as, in particular, T-cell activating agents like interleukine-2 etc.

The peptides of this invention may also be useful for other purposes, such as diagnostic use. For example, they may be used to check whether a vaccination with a peptide according to the invention has been successful. This may be done in vitro by testing whether said peptide is able to activate T cells of the vaccinated person.

In order to identify HPV16 E6 and E7 region peptides that could bind to HLA-A2.1 molecules the amino acid sequence of HPV16 E6 and E7 was examined (4). Every amino acid in the E6 and E7

region was used as the first amino acid of a 9 amino acid long peptide. In this way the entire HPV16 E6 and E7 region was covered. Nine amino acid long peptides were chosen because they fit the presently known rules for length of peptides that bind to the groove of HLA-A2.1 molecules (reviewed in 1). For practical reasons, alanine residues were used in the tested peptides instead of the cysteine residues occurring in the natural sequence.

Only the 16 peptides described in Table I (but containing alanine residues instead of cysteine residues) were able to significantly upregulate the expression of HLA-A2.1 molecules measured as mean HLA-A2.1 fluorescence of 174. CEM T2 cells indicating their binding to the HLA-A2.1 molecule as described in Example 2.

None of the 224 other peptides were able to do this. These experiments indicate that only a limited proportion of peptides (named 1-16) have the ability to bind to the HLA-A2.1 molecule and are therefore the only candidates of the HPV16 E region to be recognized by human CTL because CTL recognize peptides only when bound to HLA molecules. The following examples illustrate the present invention without limiting the same thereto.

EXAMPLE 1

Materials

Peptide synthesizer:

Abimed AMS 422 (Abimed Analysen-Technik GmbH, Langenfeld, Germany).

Synthesis polymer:

Tentagel S AC (0.17-0.24 meq/g, Rapp Polymere, Tübingen, Germany).

HPLC equipment:

The HPLC system used for analysis and purification of peptides consisted of: autosampler 2157, HPLC pump 2248, variable wavelength monitor VWM 2141, column oven 2155, low pressure mixer, all of Pharmacia Nederland B.V., Woerden,

The Netherlands, a Star LC-20 dot matrix printer, Star Micronics Co., Ltd., all parts controlled by a Tandon PCAsl/386sx computer, Tandon Computer Benelux B.V., Amsterdam, The Netherlands.

Lyophylizer:

Virtis Centry, The Virtis Company, Inc., Gardiner (NY), USA, equipped with an Alcatel 350C vacuumpump, Alcatel CIT, Malakoff, France, connected to a Christ Alpha RVC vacuo-spin, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany.

Centrifuge:

MSE Mistral 6L, Beun de Ronde, Abcoude, The Netherlands.

Mass spectrometer:

Bioion plasma desorption mass spectrometer (PDMS), Applied Biosystems, Inc., Foster City (CA), USA.

Amino acid Analysis:

HP Aminoquant, Hewlett Packard, Amstelveen, The Netherlands.

Chemicals:

All chemicals were used without further purification unless stated otherwise.

Fmoc (9-fluorenylmethyloxycarbonyl) amino acid were of the L-configuration, bearing the following side chain protecting groups: t-Bu (tert-butyl) for Asp, Glu, Tyr, Ser and Thr; Trt (trityl) for His, Asn and Gln; Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) for Arg; Boc (tert-butyloxycarbonyl) for Lys, all Novasyn and purchased from Pharmacia Nederland B.V., Woerden, The Netherlands.

Piperidine was purchased from Aldrich Chemie Benelux N.V., Brussels, Belgium.

BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate) was obtained from Richelieu Biotechnologies, St-Hyacinthe, Canada.

N-methylmorpholin (NMM, Janssen Chimica, Tilburg, The Netherlands) was distilled from NaOH at atmospheric pressure before use.

N-methylpyrrolidone (NMP, Aldrich Chemie) was vacuum-distilled under a nitrogen atmosphere (b.p. 78-80°C, 18 mm Hg) before use.
Acetonitrile (HPLC-grade) was purchased from Rathburn Chemicals Ltd., Walkerburn, Scotland.
Ether (Baker Analyzed grade), pentane (Baker grade) and acetic acid (Baker Analyzed grade) were purchased from J.T. Baker B.V., Deventer, The Netherlands.
Ethanethiol was obtained from Fluka Chemie, Brussels, Belgium.
Dichloromethane and N,N-dimethylacetamide (DMA) were purchased from Janssen Chimica, Tilburg, The Netherlands.
Trifluoroacetic acid (TFA, z.S. grade) was obtained from Merck-Schuchardt, Hohenbrunn, Germany.

Disposables:

Polypropylene reaction vessels containing a PTFE filter were purchased from Abimed Analysen-Technik GmbH, Langenfeld, Germany.
All other disposables used were made of polypropylene and obtained from Sarstedt B.V., Etten-Leur, The Netherlands.

Experimental conditions:

All experiments were performed at room temperature unless stated otherwise. All Fmoc protected aminoacids, synthesis polymers, peptides and TFA were stored at -20°C.

<u>Peptide synthesis</u>

Peptides were synthesized by solid phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) (6, 7).

The peptides were made in various runs, in each of which 48 different peptides were synthesized simultaneously.

Tentagel S AC (8, 9), a graft polymer of polyethyleneglycol spacer arms on a polystyrene matrix, was used as a resin (40-60 mg per peptide, 10 μmol Fmoc amino acid loading).

Repetitive couplings were performed by adding a mixture of 90 μl 0.67 M BOP (10, 11) in NMP, 20 μl NMM in NMP 2/1 (v/v) and

100 µl of an 0.60 M solution of the appropriate Fmoc amino acid (12) in NMP (6-fold excess) to each reaction vessel. At 70% of the reaction time approximately 50 µl dichloromethane was added to each reaction vessel.

Fmoc-deprotection was performed by adding 3 times 0.8 ml of piperidine/DMA 1/4 (v/v) to each reaction vessel.

Coupling- and deprotection times were increased as the synthesis proceeded, starting with 30 min and 3 times 3 min respectively.

Washings after couplings and Fmoc-deprotections were done with 6 times 1.2 ml DMA. After the required sequence had been reached and the last Fmoc-protection was removed the peptidylresin was washed extensively with DMA, dichloromethane, dichloromethane/ether 1/1 (v/v) and ether respectively, and dried.

Peptide cleavage and isolation

Cleavage of the peptides from the resin and removal of the side chain protecting groups was performed by adding 6 times 200 µl TFA/water 19/1 (v/v) at 5 min intervals to each reaction vessel, thus yielding free carboxylic peptides. For Trp containing peptides TFA/water/ethanethiol 18/1/1 (v/v/v) was used.

Two hours after the first TFA addition the peptides were precipitated from the combined filtrates by addition of 10 ml ether/pentane 1/1 (v/v) and cooling to -20°C. The peptides were isolated by centrifugation (-20°C, 2500g, 10 min).

After treatment of the pellet with ether/pentane 1/1 (v/v) and isolation by the same centrifugation procedure, the peptides were dried at 45°C for 15 min.

Each of the peptides was dissolved in 2 ml water (or 2 ml 10 vol.% acetic acid), the solution frozen in liquid nitrogen for 3 min, and lyophilized while being centrifuged (1300 rpm, 8-16 h).

Analysis and purification

The purity of the peptides was determined by reversed phase HPLC; an aliquot of about 50 nmol was dissolved in 100 µl 30 vol.% acetic acid. Of this solution 30 µl was applied to an RP-HPLC system equipped with a ternary solvent system; A: water, B: acetonitrile, C: 2 vol.% TFA in water.

Gradient elution (1.0 ml/min) was performed from 90% A, 5% B, 5% C to 20% A, 75% B, 5%C in 30 min. Detection was at 214 nm.

Samples taken at random were analysed by mass spectrometry on a PDMS. The 16 binding peptides were all analysed by mass spectrometry on a PDMS and by quantative amino acid analysis after hydrolysis on a HP Aminoquant. Of all analysed samples the difference between calculated and measured masses was within the experimental error (0.1%) as specified by the producer of the equipment used. All aminoacid compositions were as expected.

EXAMPLE 2

Peptides

From all 240 peptides that had been freeze dried, 5 mg was weighed and dissolved in 1 ml of distilled water. Peptides that did not readily dissolve were treated with 150 µl of 100% acetic acid glacial (CH_3COOH, Merck Darmstadt, Germany: 56-1000) after which the pH was neutralized to pH7 with 5N NaOH diluted in distilled water (Merck Darmstadt, Germany: 6498). Of all peptides a dilution of 1 mg/ml in 0.9% NaCl was made.

Cells

(174.CEM) T2 cells were cultured in Iscove's modified Dulbecco's medium (Biochrom KG Seromed Berlin, Germany: F0465) supplemented with 100IU/ml penicillin (Biocades Pharma, Leiderdorp, The Netherlands), 100 µg/ml kanamycin (Sigma St. Louis, USA:K-0254), 2mM glutamine (ICN Biomedicals Inc. Costa Mesa, CA,

USA:15-801-55) and 10% fetal calf serum (FCS, Hyclone Laboratories Inc. Logan, Utah, USA:A-1115-L). Cells were cultured at a density of 2.5 x 10^5/ml during 3 days at 37°C, 5% CO_2 in humified air.

Peptide binding

(174.CEM) T2 cells were washed twice in culture medium without FCS and put, at a density of 2×10^6 cells/ml in serum free culture medium. Of this suspension 40 µl was put into a V bottommed 96 well plate (Greiner GmbH, Frickenhausen, Germany: 651101) together with 10 µl of the individual peptide dilutions (of 1 mg/ml). The end concentration is 200 µg/ml peptide with 8 x 10^4 (174.CEM) T2 cells. This solution was gently agitated for 3 minutes after which an incubation time of 16 hours at 37°C, 5% CO_2 in humified air took place. Then cells were washed once with 100 µl 0.9% NaCl, 0.5% bovine serum albumin (Sigma St. Louis, USA:A-7409), 0.02% NaN_3 (Merck Darmstadt, Germany:822335). After a centrifuge round of 1200 rpm the pellet was resuspended in 50 µl of saturating amounts of HLA-A2.1 specific mouse monoclonal antibody BB7.2 for 30 minutes at 4°C. Then cells were washed twice and incubated for 30 minutes with $F(ab)_2$ fragments of goat anti-mouse IgG that had been conjugated with fluoresceine isothiocyanate (Tago Inc Burlingame, CA, USA: 4350) in a dilution of 1:40 and a total volume of 25 µl.

After the last incubation, cells were washed twice and fluorescence was measured at 488 nanometer on a FACScan flowcytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Figure 1

The (174.CEM) T2 cell line expresses "empty" and unstable HLA-A2.1 molecules that can be stabilized when a peptide is binding to the peptide presenting groove of these molecules. A stabilized HLA-A2.1 molecule that will not easily degrade is the result of binding of an analyzed peptide. This leads to an increase in cell surface expression of the HLA-A2.1 molecule. The

result of the binding analyses of the 240 peptides is given in Figure 1. Background fluorescence level (without adding peptides) was set on an arbitrary mean fluorescence level of 70. Binding of a peptide was regarded positive when twice the level of background fluorescence was reached. The 16 binding peptides are numbered 1 to 16.

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SEQUENCE LISTING

SEQ ID NO:1

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

LysLeuProGlnLeuCysThrGluLeu
1 5 9

SEQ ID NO:2

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

GlnLeuCysThrGluLeuGlnThrThr
1 5 9

SEQ ID NO:3

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

LeuCysThrGluLeuGlnThrThrIle
1 5 9

SEQ ID NO:4

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

GluLeuGlnThrThrIleHisAspIle
1 5 9

SEQ ID NO:5

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

LeuGlnThrThrIleHisAspIleIle
1 5 9

SEQ ID NO:6

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

ThrIleHisAspIleIleLeuGluCys
1 5 9

SEQ ID NO:7

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

IleHisAspIleIleLeuGluCysVal
1 5 9

SEQ ID NO:8

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

CysValTyrCysLysGlnGlnLeuLeu
1 5 9

SEQ ID NO:9

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

LysIleSerGluTyrArgHisTyrCys
1 5 9

SEQ ID NO:10

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

ProLeuCysAspLeuLeuIleArgCys
1 5 9

SEQ ID NO:11

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

ThrLeuHisGluTyrMetLeuAspLeu
1 5 9

SEQ ID NO:12

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

TyrMetLeuAspLeuGlnProGluThr
1 5 9

SEQ ID NO:13

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

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MetLeuAspLeuGlnProGluThrThr
  1           5           9
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SEQ ID NO:14

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

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ThrLeuGluAspLeuLeuMetGlyThr
  1           5           9
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SEQ ID NO:15

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

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GlyThrLeuGlyIleValCysProIle
  1           5           9
```

SEQ ID NO:16

SEQUENCE TYPE: amino acid

SEQUENCE LENGTH: 9 amino acids

```
ThrLeuGlyIleValAlaProIleCys
  1           5           9
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CLAIMS

1. A peptide comprising an amino acid sequence derived from a protein of human papilloma virus (HPV), wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex (MHC) Class I molecule.

2. A peptide according to claim 1, wherein said amino acid sequence is derived from protein E6 or E7 of HPV16.

3. A peptide according to claim 1, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1.

4. A peptide according to claim 2, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1.

5. A peptide according to claim 4, wherein said amino acid sequence is selected from the group consisting of:

1. KLPQLCTEL (residues 18-26 of HPV16 protein E6)
2. QLCTELQTT (residues 21-29 of HPV16 protein E6)
3. LCTELQTTI (residues 22-30 of HPV16 protein E6)
4. ELQTTIHDI (residues 25-33 of HPV16 protein E6)
5. LQTTIHDII (residues 26-34 of HPV16 protein E6)
6. TIHDIILEC (residues 29-37 of HPV16 protein E6)
7. IHDIILECV (residues 30-38 of HPV16 protein E6)
8. CVYCKQQLL (residues 37-45 of HPV16 protein E6)
9. KISEYRHYC (residues 79-87 of HPV16 protein E6)
10. PLCDLLIRC (residues 102-110 of HPV16 protein E6)
11. TLHEYMLDL (residues 7-15 of HPV16 protein E7)
12. YMLDLQPET (residues 11-19 of HPV16 protein E7)
13. MLDLQPETT (residues 12-20 of HPV16 protein E7)
14. TLEDLLMGT (residues 78-86 of HPV16 protein E7)
15. GTLGIVCPI (residues 85-93 of HPV16 protein E7)
16. TLGIVCPIC (residues 86-94 of HPV16 protein E7)
17. a fragment, homolog, isoform, derivative, genetic variant or conservative variant of any one of the amino acid

sequences Nos. 1-16 which has the ability to bind to human MHC Class I allele HLA-A2.1.

6. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

7. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5 which is able to induce a T cell response effective against HPV, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

8. A method of prophylactic or therapeutic treatment of cervical carcinoma and other HPV-related diseases with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5.

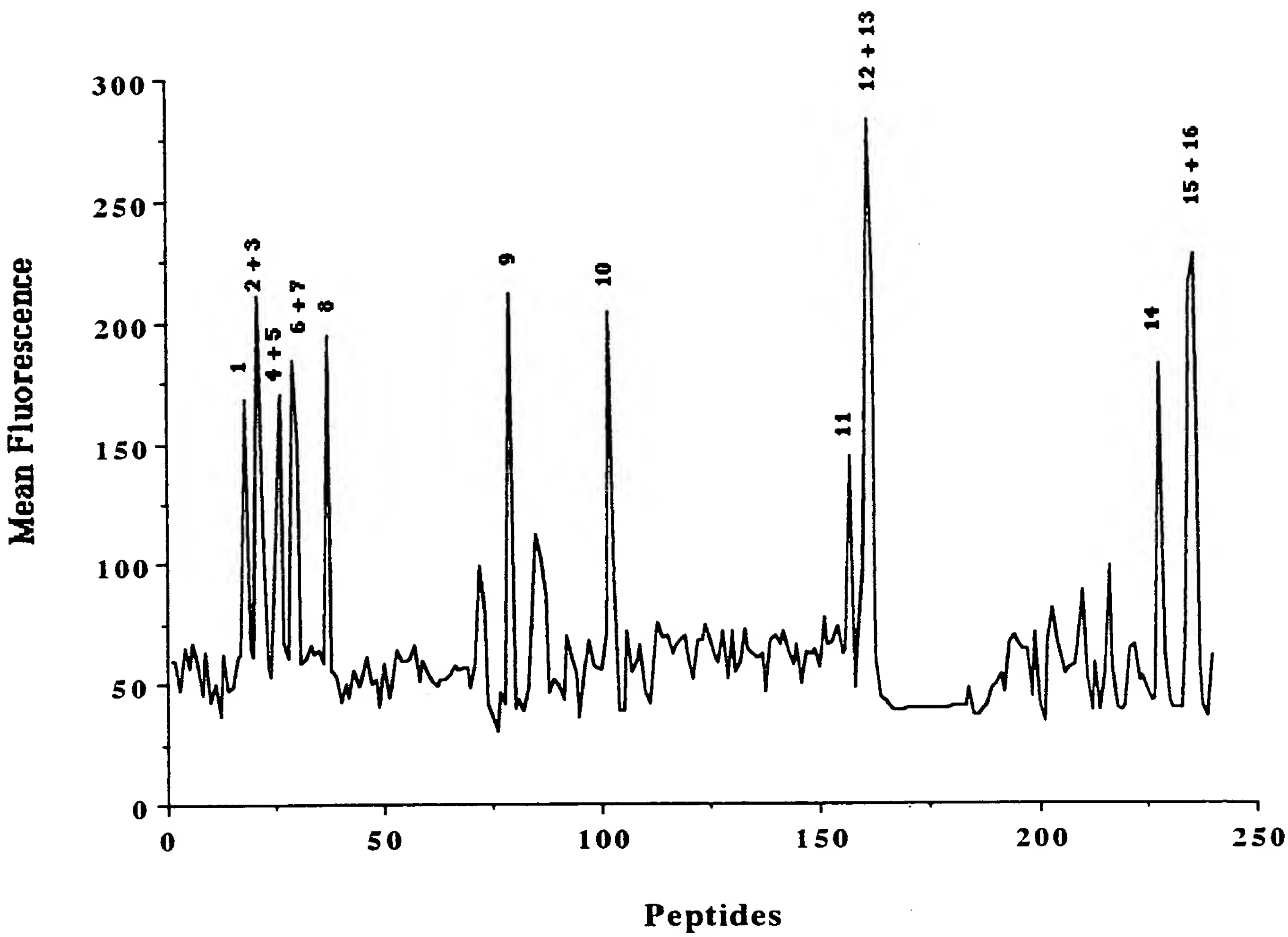
9. A method of prophylactic or therapeutic treatment of cervical carcinoma and other HPV-related diseases with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of an immunogenic form of a peptide according to any one of the claims 1-5 which is able to induce a T cell response effective against HPV.

ABSTRACT

Peptides of Human Papilloma Virus for use in human T cell response inducing compositions

A peptide comprising an amino acid sequence derived from a human papilloma virus (HPV) protein, wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex Class I molecule. Its use in prophylactic or therapeutic treatment of cervical carcinoma and other HPV-related diseases.

Binding of HPV16 E6/7 peptides to HLA A2.1



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